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Date 6/25/01 Label No. EL 706743677 US

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D B Peck

Name (Print) Signature

File No: 2901/0J410US0

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Giuseppina BESTETTI et al.

Serial No: TBA (PCT Continuation Application of International Application No. PCT/EP99/10416, filed 23 December 1999)

Filed: Concurrently Herewith

For: RECOMBINANT BACTERIAL STRAINS FOR THE PRODUCTION OF NATURAL NUCLEOSIDES AND MODIFIED ANALOGUES THEREOF

PRELIMINARY AMENDMENT (COVER SHEET)

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PRELIMINARY AMENDMENT

Hon. Commissioner of
Patents and Trademarks
Washington, DC 20231

Attn.: Box PCT, RO/US

Sir:

IN THE CLAIMS

Please cancel claims 1 through 30.

Please add the following new claims:

–31. A recombinant plasmid expression vector comprising:

a) at least one gene sequence of a mesophilic bacterium coding for a polypeptide having uridine phosphorylase enzyme activity and at least one gene sequence of a mesophilic bacterium coding for a polypeptide having purine nucleoside phosphorylase enzyme activity; and

b) at least one gene sequence coding for tetracycline and/or kanamycin resistance.

–32. A recombinant plasmid expression vector comprising:

a) at least one gene sequence of a mesophilic bacterium coding for a polypeptide having uridine phosphorylase enzyme activity or at least one gene sequence of a mesophilic bacterium coding for a polypeptide having purine nucleoside phosphorylase enzyme activity; and

b) at least one gene sequence coding for tetracycline and/or kanamycin resistance.

–33. A plasmid vector according to claim 31, wherein at least one gene sequence encoding a polypeptide having uridine phosphorylase enzyme activity, at least one gene sequence of a mesophilic bacterium coding for a polypeptide having purine nucleoside phosphorylase enzyme activity and the gene sequence coding for tetracycline and/or kanamycin resistance are cloned into the plasmid pUC18.

–34. A plasmid vector according to claim 31, wherein the mesophilic bacterium is *E.coli.*

–35. A plasmid vector according to claim 34, wherein the sequence encoding a polypeptide having uridine phosphorylase enzyme activity is the sequence *udp.*

–36. A plasmid vector according to claim 35, wherein the sequence is the EMBL sequence having accession number X15689.

–37. A plasmid vector according to claim 34, wherein the sequence encoding a polypeptide having purine nucleoside phosphorylase enzyme activity is the sequence *deoD*.

–38. A plasmid vector according to claim 37, wherein the sequence is the EMBL sequence having accession number M60917.

–39. A plasmid vector according to claim 31, wherein the sequence coding for tetracycline resistance is the Tet gene of pBR322.

–40. A plasmid vector according to claim 31, wherein the sequence coding for kanamycin resistance is the kan gene of pET29c.

–41. A plasmid vector according to claim 31, wherein said gene sequence coding for a polypeptide having uridine phosphorylase enzyme activity and said gene sequence coding for a polypeptide having purine nucleoside phosphorylase enzyme activity are fused together so to express a fusion protein wherein the enzymes uridine phosphorylase and purine nucleoside phosphorylase are covalently bonded together.

–42. A plasmid vector according to claim 31, wherein said gene sequence coding for a polypeptide having uridine phosphorylase enzyme activity and said gene sequence coding for a polypeptide having purine nucleoside phosphorylase enzyme activity are fused together so to express a fusion protein wherein the enzymes uridine phosphorylase and purine nucleoside phosphorylase are bonded together by a polypeptide linker of more than one aminoacidic units.

–43. A plasmid vector selected from those having sequence: SEQ ID NO 1, SEQ ID NO 2, SEQ ID NO 3, SEQ ID NO 4, SEQ ID NO 5, SEQ ID NO 6, SEQ ID NO 7, SEQ ID NO 8, SEQ ID NO 9, SEQ ID NO 10, SEQ ID NO 12, SEQ ID NO 13, SEQ ID NO 14 and SEQ ID NO 15.

–44. Prokaryotic host cells, which contain at least one plasmid vector according to claim 31.

–45. Host cells according to claim 44, wherein they are bacterial cells, preferably of *Escherichia coli*.

–46. Host cells according to claim 44, wherein they are cells of strain K12, preferably MG1655 or DH5α, and/or of strain B.

–47. Host cells according to claim 44, containing a plasmid vector according to claim 41.

–48. Use of host cells containing a recombinant plasmid expression vector according to claim 31 in the production of polypeptides having uridine

phosphorylase enzyme activity and/or purine nucleoside phosphorylase enzyme activity.

–49. Use of host cells containing a recombinant plasmid expression vector according to claim 31 as catalysts of transglycosylation reactions between a donor nucleoside and an acceptor base.

–50. Use according to claim 49, wherein the acceptor base is a purine and/or pyrimindine base.

–51. Use according to claim 50, wherein the purine and/or pyrimidine bases are selected from natural or substituted pyrimidine and purine bases; purine bases substituted at the 1, 2 and/or 6 positions of the purine ring; pyrimidine bases substituted at the 3 and/or 5 positions of the pyrimidine ring; purine, 2-azapurine, 8-azapurine, 1-deazapurine (imidazopyridine), 3-deazapurine, 7-deazapurine.

–52. Use according to claim 49, wherein the acceptor bases are constituted by heterocyclic compounds containing at least one nitrogen atom, such as, for example, imidazoles, triazoles and pyrazoles.

--53. Use according to claim 49, wherein the donor nucleoside is selected from natural and/or modified nucleosides containing D-ribose and 2'-deoxyribose; nucleosides containing the ribose group modified in the 2', 3' and /or 5' positions; nucleosides in which the sugar is β-D-arabinose, α-L-xylose, 3'-deoxyribose, 3',5'-dideoxyribose, 2',3'-dideoxyribose, 5'-deoxyribose, 2',5'-dideoxyribose, 2'-amino-2'-deoxyribose, 3'-amino-3'-deoxyribose, 2'-fluoro-2'-deoxyribose.

–54. Use according to claim 49 in the preparation of nucleoside containing heterocyclic systems having purine and/or pyrimidine bases substituted by one or more nitrogen atoms.

–55. Use according to claim 49 in the preparation of α-pentose-1-phosphate sugars by phosphorolysis reactions.

–56. Use according to claim 49 in the production of nucleosides.

–57. Use of the crude or purified extracts of host cells according to claim 47 as catalysts of transglycosylation reactions between a donor nucleoside and an acceptor base.

–58. A method for producing a fusion protein having the activity of both uridine phosphorylase and purine nucleoside phosphorylase enzymes, said method comprising:

a) producing a plasmid expression vector according to claim 40;

b) transforming a host bacteria cell with said expression vector; and

c) isolating and purifying the fusion protein from the transformed bacteria cell.

–59. A method according to claim 57 wherein said host bacteria cells are cells of *Escherichia coli*.

–60. A fusion protein obtainable from the method according to claim 58.

REMARKS

The claims of the application have been amended in view of the International Preliminary Examination Report, to remove multiple claim dependencies and to place them in better condition for US examination. No new subject matter has been added.

Consideration of this Amendment is respectfully requested. For the record, the cover page herein (not numbered) as well as the present page 8 were not submitted with the preliminary amendment that was submitted to the inventors along with the declaration under 37 CFR §1.63 for execution of the latter.

Respectfully submitted,

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